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Analysis of Tocopherols by High Performance Liquid Chromatography

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Abstract: Gas chromatography is the key technique for organic components and also for tocopherols analysis. High performance liquid chromatography has an important role to take part in applications such as the handling of less usual samples, prevention of degradation of heat sensitive functional groups and for micro preparative purposes. Many approaches for development of improved methods are suggested, especially for reversed phase applications.

Keywords: HPLC, Tocopherals, Chromatographic techniques, Reverse phase and Preparative.

Introduction

The key contributor for the organic samples analysis is the gas chromatography especially the flame ionization detector is vigorous and has a massive dynamic range, so accurate quantification is rarely a problem. The high performance liquid chromatography (HPLC) is a key instrument to find more about the component studies in the tocopherols.

A major advantage can be that HPLC operates at ambient temperature so there is relatively little risk to sensitive functional groups. It should also be remembered that HPLC is not merely an analytical technique, but can be used equally easily for micro preparative purposes. It is easy to collect fractions for analysis by other techniques such as by chemical degradation or by mass spectrometry (MS)[1] or nuclear magnetic resonance spectroscopy[2]. Indeed, direct analysis by HPLC-MS with electro spray ionization may offer an advantage in terms of sensitivity.

Experimental

The organic components need to extract from the raw materials before go for the analysis, the tocopherols extract from the oils like palm oil. Also the experimental step include the purification also since this study is more basic, I have used the thin layer chromatography for the purification with organic solvents like methanol and chloroform

Extraction

The cell suspension is centrifuged and the cell pellet is resuspended in 0.8 mL of 25 mM EDTA pH 7 (to prevent phospholipid degradation). Total lipids are extracted by vortexing 20 min after addition of 1 mL chloroform, 1 mL methanol and 50 µl of 15 mM BHT, and again 5 min after further additions of 1 mL chloroform and 1 mL NaCl to obtain two phases. After centrifugation, the lower phase is washed if necessary with 2 mL of 1 M NaCl and evaporated under nitrogen.

Purification

Tocopherols, cholesterol and individual phospholipids are separated by one dimensional TLC on Whatman LK5 silica gel plates. Plates were previously washed by migration up to the top in chloroform/methanol (1/1) in a special clean tank, dried and largely wetted with a solution of 2.5% boric acid in ethanol (except the concentration zone). After draining and dessication at 100 °C (15 min), samples are applied as streaks in the concentration zone. The solvent system used is the same we have described for phospholipids but with addition of 10 mg/mL ascorbic acid (previously dissolved in water) and BHT (0.15 mg/mL) to protect tocopherols and unsaturated fatty acids against oxidation. The plates are developed up to 1 cm below the top in a dark chamber (the glass tank is under a plastic box). After drying, lipid spots are located under a UV lamp after a primulin spray (1 mg of primuline powder in 100 mL of a mixture acetone/water, 80/20). Commercial standard are used to locate the lipids of interest. Tocopherols and cholesterol have a quite similar migration and are found near the solvent front (average RF: 0.87).

The spot is scraped off and tocopherols eluted by vortexing some minutes with 3 mL of a mixture hexane/*ter*-butyl ether (92/8) or with pure *ter*-butyl ether if cholesterol must also be determined. The solvent phases are evaporated under nitrogen and the residue, dissolved in methanol, is stored in amber glass ware at -20 °C.

Analysis

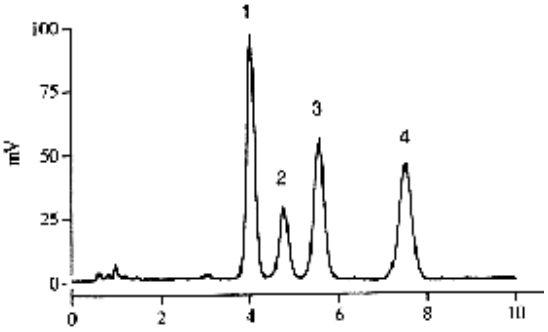
Tocopherols are separated on a column packed with 5 µm LiChrosorb RP18 (Merck) (120 x 4.6 mm), with methanol/water (98/2) as the mobile phase, flow rate: 1.5 mL/min.

Detection

Tocopherols can be detected by light scattering, spectrophotometry, fluorimetry and electrochemistry (cholesterol can be detected by light scattering and spectrophotometry). Tocopherol acetate (commonly used in food, dietetic and pharmaceutical preparations) is not fluorescent and may be detected by UV photometry. As response factors are different for the different tocopherols with any detector system, standard curves must be determined for each component.

With UV detection (292 nm for free tocopherols and 284 nm for tocopherol acetate), the minimum detection limit is about 15-30 ng (35-70 pmol) per injection. With fluorescence detection (excitation at 292 nm, emission at 327 nm) the minimum detection limit is about 0.5 ng (2-3 nmol) according to the instrument used. With the evaporative light-scattering detector, responses are linear in the range 0.1-0.5 µg and the minimum detection limits are about 10-20 ng per injection. These observations were made with a DDL21 detector (Eurosep Instruments) optimized with an air pressure set at 1 bar, an evaporation temperature at 60 °C and a high voltage at 700 V. Other instruments should be optimized specifically. δ-Tocopherol, rarely present in animal tissues and tocopherol acetate are often

used as internal standards to correct from losses during extraction and washings. Several ways to perform sample pretreatment and analysis of α-tocopherol may be found[3] by many people. Also it includes referenced tables providing in-depth summaries of methodology for the analysis of α-tocopherol and related compounds in foods, pharmaceuticals, plants, animal tissues and other matrices. A typical chromatogram of a mixture of tocopherols obtained with a light scattering detector is given below.



Time in minutes

Peaks :1) δ-Tocopherol, 2) γ-Tocopherol, 3) α-Tocopherol, 4) Tocopherol acetate.

Figure 1. A typical chromatogram of a mixture of tocopherols

Results and Discussion

A comparison of chromatographic separation of tocopherols by reversed-phase, normal-phase, and gas chromatography were established[4].Several methods have been developed for simultaneously determining α-tocopherol and cholesterol since they have both important dietary functions and furthermore share similar chromatographic properties. With the HPLC system described above, cholesterol eluted after about 9.5 min and is easily quantified with a light-scattering detector.

Another chromatographic system has been described involving a silica column eluted with an isocratic mobile phase made[5] of isooctane/tetrahydrofuran (90/10, v/v). A fluorescence detector was used to quantify tocopherols and a light-scattering detector to quantify cholesterol. Fluorescence was preferably used since, in the absence of previous purification of muscle extracts, this technique prevents interferences from unknown non-fluorescent substances eluting near α-tocopherol.

Very good and rapid separations of tocopherols were also obtained using nano-HPLC with a capillary column (100 μm ID) filled with 3 μm RP18 particles[6] .Monolithic column were shown to improve the determination of vitamin E and A in human serum in shortening the time of analysis four times in comparison with using traditional particulate columns. An example of simultaneous determination of α-tocopherol, β-carotene and retinol by HPLC with diode-array detection may be found in the work[7,8]. The precise analysis of the carboxyethyl-hydroxychroman metabolites of α-and γ-tocopherol found in plasma or urine is possible when using a combination of gas chromatography with mass spectrometry[9].

Conclusion

The study which present here is just a simple study but the HPLC technique improve a lot and touch a remarkable place in the organic components analysis. There is no doubt about that, the further studies in the HPLC will lead us towards a wide spectrum in the field of analytical and synthetic organic chemistry.

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